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Molecular Evidence that *Sclerolinum brattstromi* Is Closely Related to Vestimentiferans, not to Frenulate Pogonophorans (Siboglinidae, Annelida)

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Abstract. Siboglinids, previously referred to as pogonophorans, have typically been divided into two groups, frenulates and vestimentiferans. Adults of these marine protostome worms lack a functional gut and harbor endosymbiotic bacteria. Frenulates usually live in deep, sedimented reducing environments, and vestimentiferans inhabit hydrothermal vents and sulfide-rich hydrocarbon seeps. Taxonomic literature has often treated frenulates and vestimentiferans as sister taxa. *Sclerolinum* has traditionally been thought to be a basal siboglinid that was originally regarded as a frenulate and later as a third lineage of siboglinids, Monilifera. Evidence from the 18S nuclear rDNA gene and the 16S mitochondrial rDNA gene presented here shows that *Sclerolinum* is the sister clade to vestimentiferans although it lacks the characteristic morphology (*i.e.*, a vestimentum). The rDNA data confirm the contention that *Sclerolinum* is different from frenulates, and further supports the idea that siboglinid evolution has been driven by a trend toward increased habitat specialization. The evidence now available indicates that vestimentiferans lack the molecular diversity expected of a group that has been argued to have Silurian or possibly Cambrian origins.

Introduction

Siboglinids were formerly called pogonophorans and include two groups of marine protostomes, frenulates and vestimentiferans, that are commonly referred to as beardworms and tubeworms, respectively. Both groups lack a functional gut as adults and rely on endosymbiotic bacteria for nutrition. They have a closed circulatory system and possess a metamerized tail region called the opisthosoma. Vestimentiferans are distinguished from frenulates by the presence of a vestimentum, a winged region near the anterior of the organism. Both taxa occur in reducing environments and typically are found at depths below several hundred meters. Due to the limited availability of samples and the difficulty of retrieving live specimens, several aspects of their biology (*e.g.*, reproduction, physiology) are still poorly understood. Vestimentiferans, in general, have been better studied than frenulates because they are keystone species in eastern Pacific hydrothermal vent habitats and in Pacific and Caribbean seeps.

The taxonomic literature concerning frenulate and vestimentiferan siboglinids has a colorful and confusing history. One taxonomic scheme recognizes frenulates (aka pogonophorans *sensu stricto*) and vestimentiferans as distinct phyla (Jones, 1985). Alternatively, vestimentiferans have also been recognized as a class within the phylum Pogonophora (Jones, 1981; Ivanov, 1994). Others place frenulates and vestimentiferans within the phylum Annelida (Land and Nørrevang, 1977; Kojima *et al.*, 1993; Bartolomaeus, 1995; McHugh, 1997; Rouse and Fauchald, 1997; also see Southward, 1988). The latter hypothesis has been supported by recent morphological (Rouse and Fauchald, 1995, 1997), embryological (Young *et al.*, 1996; Southward, 1999), and molecular analyses (Kojima *et al.*, 1993; McHugh, 1997; Black *et al.*, 1997; Kojima, 1998; Halanych *et al.*, 1998). To further complicate matters, a ranked classification scheme has produced different names for the same clade of organ-

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isms. Vestimentiferans have been called Vestimentifera (Jones, 1981), Obturata (Jones, 1981; Southward, 1988; Southward and Galkin, 1997), and Afrenulata (Webb, 1969). Frenulates have been called Pogonophora (Jones, 1985), Frenulata (Webb, 1969), Perviata (Southward, 1988), and originally Siboglinidae (Caullery, 1914).

Hereafter we apply the following nomenclature: (1) Vestimentifera are equated with Obturata and Afrenulata; (2) Frenulata are equated with Perviata and Pogonophora (*sensu* Jones, 1985); (3) Monilifera is a third monogeneric clade that includes *Sclerolinum;* and (4) Siboglinidae refers to the clade that includes Vestimentifera, Frenulata, and Monilifera. We recognize that the term "Pogonophora" is more commonly used and that rules of priority for nomenclature do not apply to higher taxa. However, we have opted to use the term "Siboglinidae" throughout this manuscript to emphasize that this group of organisms represents derived annelids (McHugh, 1997; Rouse and Fauchald, 1997). We restrict the term "pogonophoran" to common usage.

Even among siboglinids, there has been one group, *Sclerolinum,* that has been particularly problematic in terms of phylogenetic position. Unlike most frenulates that live in the mud, *Sclerolinum* species can live on decaying organic material like wood or rope made from natural fibers (Webb, 1964a; Southward, 1972). This taxon was originally considered a member of the frenulate family Polybrachiidae (Southward, 1961), but Webb (1964b), mainly citing differences in the postannular region, argued that *Sclerolinum* could not be ascribed to either of the two orders (Thecanephria and Athecanephria) of siboglinids recognized at the time (vestimentiferans had not been discovered yet). He erected a new family, Sclerolinidae, that he states should "have order rank." Ivanov (1991) more formally recognized the unique nature of *Sclerolinum,* and in 1994 he proposed that Frenulata (= Perviata), Monilifera (= Sclerolinidae), and the Vestimentifera be regarded as three taxa with equal rank (*i.e.,* classes within the phylum Pogonophora). Additionally, Ivanov (1994) further suggested that Monilifera are allied to the Vestimentifera on the basis of the common absence of several characters (*e.g.,* spermatophores, telosomal diaphragm, metasoma preannular and postannular regions) relative to the Frenulata. Southward (1999) suggested that Monilifera might be similar to the ancestral siboglinid form, thus predicting that it should occupy a basal position in siboglinid phylogeny. Distinguishing between these hypotheses on the placement of *Sclerolinum* will allow us to test the notion of Black *et al.* (1997) that habitat preference or specificity may be an important factor in siboglinid evolution. If Black *et al.* are correct, *Sclerolinum* is expected to occupy a position between frenulates and vestimentiferans (which may be consistent with Ivanov's ideas), and not a position basal to the frenulate-vestimentiferan clade.

To date, molecular studies that include siboglinids have either focused on vestimentiferans (Williams *et al.,* 1993; Black *et al.,* 1997; Kojima *et al.,* 1997; Halanych *et al.,* 1998) or have addressed siboglinid origins (Winnepenninckx *et al.,* 1995a; Kojima *et al.,* 1993; Kojima, 1998; McHugh, 1997). Most studies have included only one frenulate representative. Although Black *et al.* (1997) included two "frenulate" siboglinids, one of these, the Loihi worm, was undescribed. Additionally, several 18S sequences were reported in a symposium contribution (Halanych *et al.,* 1998) for which page limitations did not permit detailed analyses or explanation. Herein we extend these previous analyses by increasing the sampling of frenulates, including *Sclerolinum,* and using novel 18S rDNA and 16S rDNA data. The present findings support the notion that habitat requirements have been important in siboglinid evolution. Additionally, frenulates are sister to a *Sclerolinum*-vestimentiferan clade, the latter of which showed limited diversity suggestive of a recent radiation within the clade.

Materials and Methods

Taxa employed

Table 1 lists the species analyzed and GenBank accession numbers for the rDNA sequences used in this study. The frenulate and vestimentiferan operational taxonomic units (OTUs) included in this study represent all of the currently recognized genera available to the authors. The addition of closely related species within a genus would have increased OTUs without increasing the phylogenetic signal for the issues under examination and were therefore excluded. For example, there are no nucleotide differences observed in the 18S rDNA of *Escarpia spicata* (Guaymas Basin) and *E. laminata* (Florida Escarpment). Limiting the number of OTUs also reduced computation time, allowing for more thorough analyses. Unless otherwise noted, collection localities correspond to those given in Black *et al.* (1997). *Siboglinum ekmani, S. fiordicum,* and *Sclerolinum brattstromi* were collected near Bergen, Norway, and identified by Eve Southward, Marine Biological Association of the United Kingdom. Identification of the frenulates *Spirobrachia* and *Polybrachia* were made by Eve Southward on the basis of animal and tube morphology. Both specimens were collected by TVGrab from the Aleutian Trench (57°27.394′N, 148°00.013′W) at a depth of 4890 m on the German research vessel *Sonne.*

The non-siboglinid annelid OTUs for the 18S data were chosen to represent a diversity of lineages for which sequences were available. The arthropod (*Artemia*) sequence was designated as the most distant outgroup for rooting purposes. Based on both morphology (*e.g.,* Eernisse *et al.,* 1992) and molecular studies (*e.g.,* Halanych *et al.,* 1995; Winnepenninckx *et al.,* 1995a; Aguinaldo *et al.,* 1997; Eernisse, 1997), arthropods are clearly outside of the proto-

TABLE 1

Taxa used in rDNA analyses

Organism	GenBank Accession[a] 18S rDNA	16S rDNA
Pogonophora		
Frenulata		
Galathealinum brachiosum	AF168738	AF315040
Polybrachia sp.	AF168739	AF315037
Siboglinum fiordicum GB	X79876[b]	
Siboglinum fiordicum	AF315060	AF315039
Siboglinum ekmani	AF315062	AF315038
Spirobrachia sp.	AF168740	AF315036
Vestimentifera		
Escarpia spicata	AF168741	AF315041
Escarpiid n. sp.		AF315053
Lamellibrachia barhami	AF168742	AF315043
		AF315044
		AF315045
		AF315047
Oasisia alvinae	AF168743	AF315052
Ridgeia piscesae	AF168744	AF315048
		AF315051
		AF315054
Ridgeia piscesae GB	X79877[b]	
Riftia pachyptila	AF168745	AF315049
		AF315050
Tevnia jerichonana	AF168746	AF315042
Monilifera		
Sclerolinum brattstromi	AF315061	AF315046
Annelida		
Alvinellidae		
Paralvinella palmiformis	AF168747	
Chaetopterida		
Chaetopterus variopedatus	U67324[c]	
Hirudinea		
Haemopis sanguisuga	X91401[d]	
Hirudo medicinalis		AF315058
Oligochaete		
Enchytraeus sp.	Z83750[d]	
Phyllodocida		
Glycera americana	U19519[e]	
Polynoidea		
Lepidonotopodium fimbriatum		AF315056
Branchipolynoe symmytilida		AF315055
Sabellida		
Sabella pavonina	U67144[c]	
Tubificidae		
Tubifex sp.		AF315057
Echiura		
Ochetostoma erythrogrammon	X79875[b]	
Urechis sp.		AF315059
Sipuncula		
Phascolosoma granulatum	X79874[b]	
Nemertea		
Lineus sp.	X79878[b]	
Mollusc		
Scutopus ventrolineatus	X91977[f]	
Priapulida		
Priapulus caudatus	X80234[g]	
Arthropod		
Artemia salina	X01723[h]	

[a] Unless otherwise noted, sequences were obtained in this study.
[b] Sequence from Winnepenninckx *et al.* (1995a).
[c] Sequence from Nadot and Grant (unpublished).
[d] Sequence from Kim *et al.* (1996).
[e] Sequence from Halanych *et al.* (1995).
[f] Sequence from Winnepenninckx *et al.* (1996).
[g] Sequence from Winnepenninckx *et al.* (1995b).
[h] Sequence from Nelles *et al.* (1984).

stome worm radiation. Because siboglinids are not closely related to molluscs and because of rate heterogeneity problems within the Mollusca, only a single representative (the aplacophoran *Scutopus*) was used. Due to alignment limitations, outgroups employed in the 16S analyses—a leech, an oligochaete, two polynoid polychaetes, and an echiurid—were more limited (see Table 1). Because different investigators collected the data at different times, there was not a 1:1 correspondence in OTUs between data sets. We felt it more important to present all the relevant data rather than trim taxa from the data sets. The aligned data sets are available at the journal's Supplement's page (http://www.mbl.edu/BiologicalBulletin/VIDEO/BB.video.html) and at TREEBASE (http://phylogeny.harvard.edu/treebase).

Data collection

Total genomic DNA was extracted using a modified hexadecyl-trimethyl-ammonium bromide (CTAB) protocol (Doyle and Dickson, 1987). The entire 18S nuclear rDNA gene was amplified *via* PCR (polymerase chain reaction), using the universal metazoan oligonucleotide primers 18e and 18P (Halanych *et al.*, 1998). A region of the 16S mitochondrial rDNA was amplified using 16Sar-5′ and 16Sbr-3′ primers (Palumbi, 1996). Each 50 μl reaction consisted of about 50 ng of template DNA, 0.5 μM of each primer, 2.5 mM $MgCl_2$, 200 μM dNTPs, 5 μl of manufacturer's 10× reaction buffer, and 1.5 U *Taq* polymerase (Promega Inc., Wisconsin). Cycling profiles were as fol-

lows: 18S—initial denaturation at 95 °C for 3 min, 35 cycles of amplification (denaturation at 95 °C for 1 min, annealing at 50 °C for 2 min, extension at 72 °C for 2 min 30 s), and a final extension at 72 °C for 5 min; 16S—initial denaturation at 94 °C for 2 min, 40 cycles of amplification (denaturation at 94 °C for 30 s, annealing at 46 °C for 30 s, extension at 72 °C for 1 min), and a final extension at 72 °C for 7 min. PCR products were purified using the QIAEX II gel extraction kit (Qiagen Inc., California). Approximately 60 ng of purified PCR product was used in sequencing reactions according to the manufacturer's instructions (FS Dye Termination Mix or Big Dye, Applied Biosystems Inc., California). The reaction profile was 25 repetitions of denaturation at 94 °C for 30 s, annealing at 50 °C for 15 s, and extension at 64 °C for 4 min. Dye-labeled fragments were separated by electrophoresis on a Perkin Elmer ABI 373A or 377 DNA sequencer. Both strands of the PCR product were sequenced. In addition to the PCR primers, the oligonucleotide primers used for sequencing are given in Halanych *et al.* (1998) or Hillis and Dixon (1991). The sequences were assembled and verified using the AutoAssembler and Sequence Navigator programs (Applied Biosystems Inc., California). The terminal primer regions were not included in the sequences submitted to GenBank or in the phylogenetic analyses.

Phylogenetic analyses

Sequence alignment was produced with a Clustal W program (Thompson *et al.*, 1994) and subsequently corrected by hand using the protostome secondary structure models available through the Ribosomal Database project (http://rdp.cme.msu.edu/html/). Regions that could not be unambiguously aligned (*e.g.*, divergent loop domains) were excluded from analyses. Tree reconstructions were implemented with the PAUP* 4.0b4b2 program (Swofford, 2000), and MacClade 3.06 (Maddison and Maddison, 1992) was used for character and tree analyses. Neighbor-joining (NJ), parsimony, and maximum likelihood (ML) analyses were performed and yielded similar results. In the interest of brevity, results and discussion will focus on ML analyses.

NJ trees were reconstructed under Jukes-Cantor, Kimura-2-parameter, Tamura-Nei, general-time-reversible, and log/det models. All except log/det were examined under equal rates of among-site rate variation using the empirically derived gamma shape parameter, α, of 0.3 (see Swofford *et al.*, 1996, for summary of different assumptions used in these models). A Kishino-Hasegawa (1989) likelihood evaluation of the resulting topologies revealed no significant differences between models for either the 16S or the 18S data. Kishino-Hasegawa evaluations estimated a six-substitution-type rate matrix for which nucleotide base frequencies were set to empirical values and α was estimated. NJ bootstraps consisted of a log/det correction (model was arbitrarily chosen) with 1000 iterations. Parsimony analyses consisted of heuristic searches with 100 random sequence additions and tree-bisection-reconnection (TBR) branch swapping. Transitions (Ti) and transversions (Tv) were given equal weighting. ML evaluation of parsimony topologies was the same as for NJ topologies. One thousand iterations were used for parsimony bootstrap analyses. When using likelihood to search for the "best" tree (as opposed to evaluating given trees), computation time was limiting. Therefore, we used a nucleotide model with two substitution types where the Ti/Tv ratio was set to the value estimated for the best parsimony tree (empirical base frequencies were used). ML searches were heuristic with 10 random sequence-addition replicates. ML bootstraps employed the "Faststep" option with 100 iterations.

Results

The 18S rDNA data set consisted of 26 OTUs and 1935 nucleotide positions. Of the 1614 nucleotide positions that could be unambiguously aligned, 34.6% (559 positions) were variable and 18.7% (303 positions) were parsimony informative. Figure 1 shows the single best likelihood tree (Ln likelihood = −8260.55148) recovered. All search methods in all analyses found a monophyletic siboglinid clade (bootstrap support was ≥98% for all methods). Resolution within the vestimentiferan clade, as well as between annelid groups, was poor, however. The moniliferan *Sclerolinum brattstromi* falls out with the vestimentiferan taxa in all analyses (bootstrap ≥ 98%). The remaining frenulates form a distinct sister-clade to the *Sclerolinum*-vestimentiferan clade with ≥99% bootstrap support.

Resolution among annelid taxa and within the vestimentiferans was poor due to the lack of phylogenetic signal. Because this paper does not focus on the annelid radiation, we did not try to enhance resolution among all annelid taxa. However, we did attempt to boost the signal within the vestimentiferan clade by employing a less inclusive taxonomic alignment. For metazoan 18S sequences, inclusion of broader taxonomic diversity can often create larger regions of ambiguous alignment that should not be included in analyses, due to poor assumptions about positional homology. Thus by reducing the taxonomic breadth examined, the phylogenetic signal can potentially be increased by a "better" alignment (Halanych, 1998). Unfortunately, even when just the siboglinids were aligned, little genetic diversity was observed, and the vestimentiferan taxa were still poorly resolved (not shown). The exception was *Lamellibrachia barhami*, which was consistently placed as the most basal vestimentiferan. Table 2 shows the logdet/paralinear distances (below diagonal) and absolute distances (above diagonal) for this less-inclusive, siboglinid-only alignment (in which most divergent domains could be unambiguously aligned). Even though the distance values for the siboglinid-

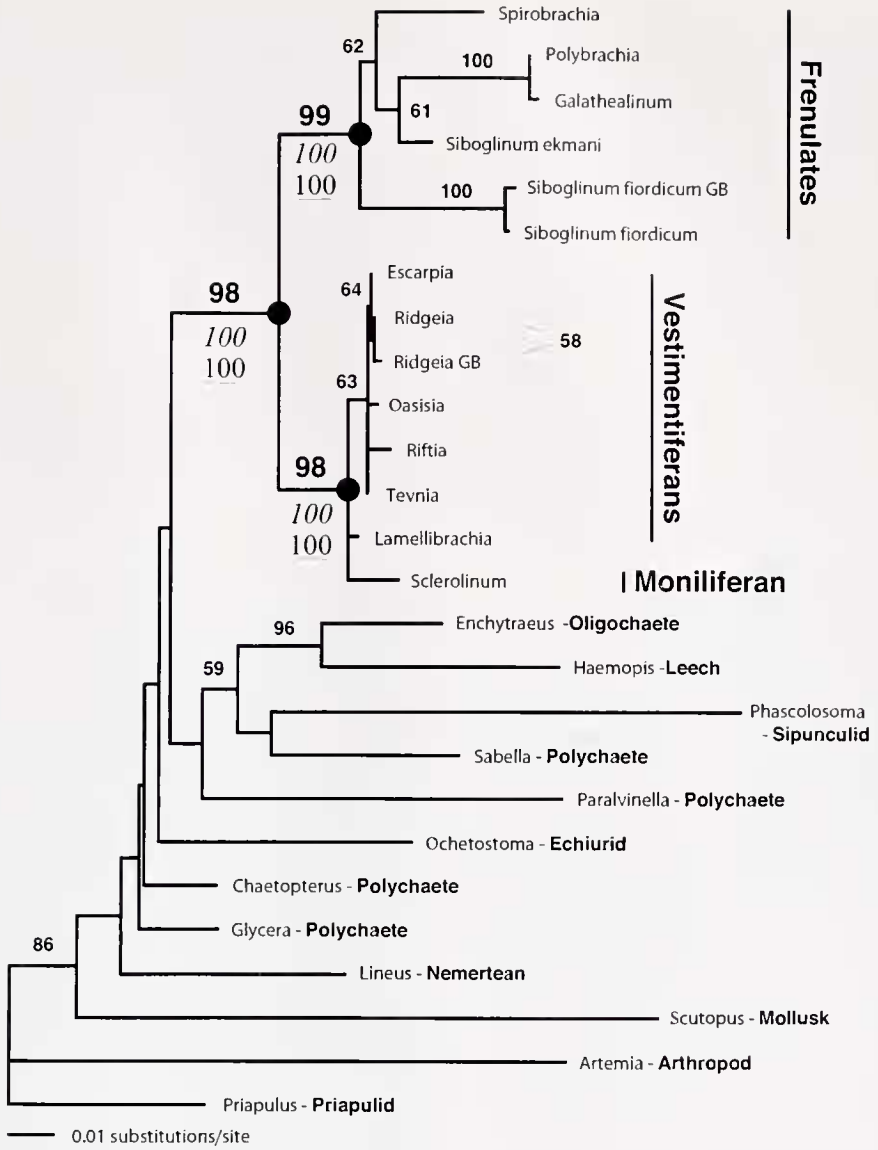

Figure 1. Results of 18S rDNA phylogenetic analyses. The single best likelihood tree (Ln likelihood = −8260.55148) found. Analysis details are given in the text. Maximum likelihood bootstrap values of ≥50% are given in bold. Parsimony (italic) and neighbor joining (underlined) values are also given for the major nodes of interest (values for other nodes were omitted in the interest of space). Branch lengths are drawn proportional to the inferred amount of change along the branch (scale shown).

only alignment are only slightly greater than the full alignment values, the greatest distance within vestimentiferans was only 0.02 (with a maximum of 25 nucleotide differences), revealing that there was very little 18S genetic diversity within this group.

The 16S rDNA data set consisted of 24 OTUs, each with 497 nucleotide positions. Of the 465 nucleotide positions that could be unambiguously aligned, 60.4% (281 positions) were variable and 47.7% (222 positions) were parsimony informative. The reconstructed topology (Ln likelihood = −3967.21062), Figure 2, was qualitatively similar to the 18S topology. Siboglinids are divided into two major lineages: vestimentiferans plus the moniliferan *Sclerolinum brattstromi* (bootstrap support 83% for ML and 100% for NJ and parsimony) and a frenulate sister-clade (bootstrap support ≥94% in all analyses). Again, *S. brattstromi* was basal to the vestimentiferans. In a departure from the 18S analyses, *Riftia pachyptila,* not *Lamellibrachia barhami,* often fell out as the most basal vestimentiferan. However, this was never supported by >54% bootstrap support; ML analyses that excluded the non-siboglinid outgroups revealed that the base of the Vestimentifera was poorly resolved with 16S data. A comparison of genetic divergence values (Table 3) indicates that there was limited genetic

TABLE 2

Pairwise distances for the siboglinid-only 18S rDNA data set; absolute distances above diagonal and log/det distances below diagonal

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *Spirobrachia*	—	109	113	87	132	131	124	122	125	124	131	125	121	120
2 *Polybrachia*	0.07	—	9	104	138	137	139	140	140	140	147	142	140	136
3 *Galathealinum*	0.07	0.01	—	106	142	141	142	143	143	143	150	145	143	139
4 *Siboglinum ekmani*	0.05	0.06	0.06	—	116	117	113	110	113	110	121	112	107	112
5 *Siboglinum fiordicum*	0.08	0.09	0.09	0.07	—	5	140	136	136	140	143	138	134	139
6 *Siboglinum fiordicum* GB	0.08	0.08	0.09	0.07	0.00	—	143	139	139	143	146	141	137	140
7 *Escarpia*	0.08	0.09	0.09	0.07	0.09	0.09	—	7	14	10	19	6	13	31
8 *Ridgeia*	0.08	0.09	0.09	0.07	0.09	0.09	0.00	—	8	7	17	4	12	32
9 *Ridgeia* GB	0.08	0.09	0.09	0.07	0.09	0.09	0.01	0.00	—	14	21	11	19	38
10 *Oasisia*	0.08	0.09	0.09	0.07	0.09	0.09	0.01	0.00	0.01	—	20	7	14	32
11 *Riftia*	0.08	0.09	0.09	0.07	0.09	0.09	0.01	0.01	0.01	0.01	—	16	25	39
12 *Tevnia*	0.08	0.09	0.09	0.07	0.09	0.09	0.00	0.00	0.01	0.00	0.01	—	11	30
13 *Lamellibrachia*	0.07	0.09	0.09	0.07	0.08	0.09	0.01	0.01	0.01	0.01	0.01	0.01	—	28
14 *Sclerolinum*	0.08	0.09	0.09	0.07	0.09	0.09	0.02	0.02	0.02	0.02	0.02	0.02	0.02	—

variation within vestimentiferans (≤0.11 log/det distance; a maximum of 47 nucleotide differences).

As for the frenulate clade, neither 18S or 16S supported a monophyletic *Siboglinum;* but because only two *Siboglinum* species were examined, additional taxa are needed to verify the status of this frenulate taxon. Additionally, we performed Kishino-Hasegawa (1989) likelihood evaluation for both genes to test the monophyly of the frenulate and vestimentiferan-*Sclerolinum* clades. To this end, we used the constraints option in PAUP* 4.0b4b2 to conduct parsimony heuristic searches (specifics same as above) to find the best trees that were consistent and inconsistent with the monophyly of these clades. Both the 16S and the 18S data significantly support the monophyly of both groups (18S frenulates—average ML score supporting monophyly = −8244.69, non-monophyly score = −8278.135, P value < 0.01; 16S frenulates—monophyly = −3894.889, non-monophyly = −3927.49, P value < 0.005; 18S vestimentiferan-*Sclerolinum*—monophyly = −8244.69, non-monophyly = −8271.922, P value < 0.05; 16S vestimentiferan-*Sclerolinum*—monophyly = −3894.889, non-monophyly = −3911.802, P value < 0.05).

Discussion

The monophyly of siboglinids (aka, Pogonophora *sensu latu*) is supported by morphological (Southward, 1988, 1993; Rouse and Fauchald, 1995; Rouse, 2001), embryological (Southward, 1999), and molecular (Winnepenninckx *et al.*, 1995a; Black *et al.*, 1997; McHugh, 1997; Halanych *et al.*, 1998, this study) evidence. Thus, in agreement with others (Southward, 1988, 1999; Ivanov, 1994; McHugh, 1997), we see no support for the recognition of vestimentiferans and frenulates as having fundamentally different body plans (*i.e.*, "phyla" *sensu* Jones, 1985). The assertion made by Webb (1964b) and later by Ivanov (1991, 1994) that *Sclerolinum* was notably different from frenulates is validated by the present data. Moreover, we found that *Sclerolinum brattstromi* is closely allied to the vestimentiferans, and does not occupy a position basal to a frenulate-vestimentiferan clade, confirming Ivanov's (1991; 1994; Ivanov and Selivanova, 1992) ideas that moniliferans occupy a position intermediate between vestimentiferans and frenulates.

Southward (1993) also suggested a possible evolutionary link between *Sclerolinum* and vestimentiferans. This contention is confirmed by the present analysis, as well as a recent morphological cladistic analysis (Rouse, 2001). Using 44 morphological characters coded for all recognized siboglinid genera, Rouse found support for the monophyly of Frenulata, Vestimentifera, and the *Sclerolinum*-vestimentiferan clade. However, our use of nomenclature differs from Rouse with regard to the term Monilifera, which he applies to the *Sclerolinum*-vestimentiferan clade. Because this term was originally (Ivanov and Selivanova, 1992) applied to only *Sclerolinum,* and because of the morphological differences from vestimentiferans, Rouse's use of the term will inject confusion into the literature. Although we acknowledge that Monilifera, as defined here, is redundant with the generic name *Sclerolinum,* several aspects of siboglinid evolution and taxonomy are in need of additional study. Thus, we have chosen not to name this clade until more is understood about siboglinid evolution.

The placement of *Sclerolinum* was especially interesting in the context of the evolution of habitat preference. Previous studies of vestimentiferans (Black *et al.*, 1997), clams (Peek *et al.*, 1997), mussels (Craddock *et al.*, 1995), and shrimp (Shank *et al.*, 1999) reveal that vent-endemic organisms are related to, and possibly derived from, species associated with hydrocarbon seeps that occur near subduction zones and continental margins. Furthermore, recent observations (Feldman *et al.*, 1998; Baco *et al.*, 1999; Distel

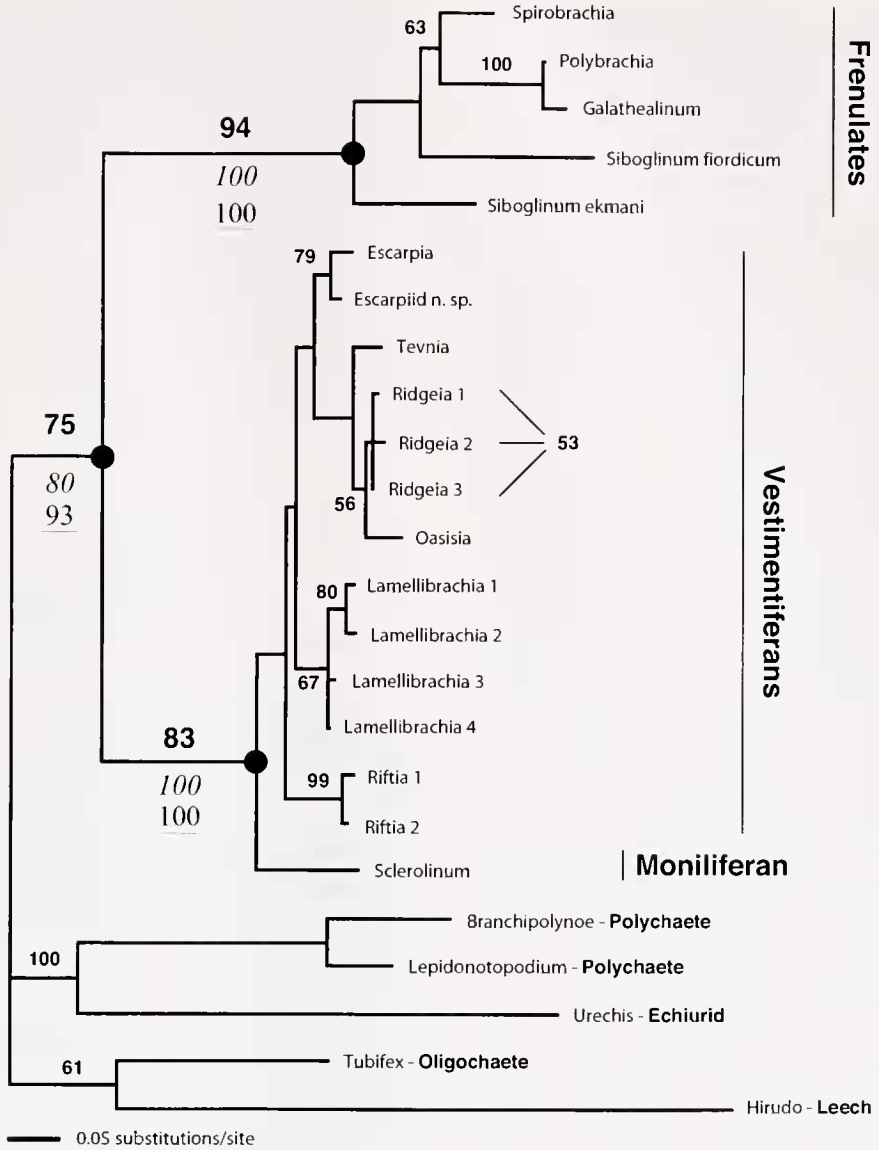

Figure 2. Results of 16S rDNA phylogenetic analyses. The best likelihood tree (Ln likelihood = −3967.21062) found. Another tree with a Ln likelihood score of −3967.25739 was found in the same search. The trees differed in relationships within the *Ridgeia* clade. Analysis details are given in the text. Maximum likelihood (ML) bootstrap values of ≥50% are given in bold. Parsimony (italic) and neighbor joining (underlines) values are also given for the major nodes of interest (values for other nodes were omitted in the interest of space). In the ML bootstrap analysis, *Lamellibrachia* and *Sclerolinum* formed a clade in 55% of the iterations. That is not shown above because it is incompatible with the "best" ML tree. Branch lengths are drawn proportional to the inferred amount of change along the branch (scale shown).

et al., 2000) reveal that several symbiont-bearing clams, vestimentiferan tubeworms, and mussels can survive on rotting organic material, such as wood or a whale carcass. The moniliferan *S. brattstromi* and related species (*e.g., S. javanicum, S. minor,* and *S. major*) are typically found growing on decaying organic material such as wood or rope (Webb, 1964a, b; Southward, 1972; Ivanov and Selivanova, 1992). Other members of the genus, (*e.g., S. sibogae* and *S. magdalenae*) lived buried in mud (Southward, 1972). These habitat preferences suggest that affinity for a mud or silt habitat was ancestral in siboglinids, allowing us to speculate that a pattern of evolution from low-oxygen, sedimented habitats to decaying organic material to hydrocarbon seeps to hydrothermal vents has occurred within the *Sclerolinum*-vestimentiferan clade.

Although neither the 18S nor the 16S data clearly resolve relationships within the Vestimentifera, the cytochrome *c* oxidase subunit I (COI) data of Black *et al.* (1997) show

TABLE 3

Pairwise distances for 16S rDNA data set; absolute distances above diagonal and log/det distances below diagonal

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 *Spirobrachia*	—	41	52	65	65	119	116	114	112	116	111	114	119	121	121	117	118	122	120	129	125	141	123	141
2 *Polybrachia*	0.09	—	8	68	67	113	111	108	108	108	106	113	114	111	113	109	106	106	117	126	114	129	121	134
3 *Galathealinum*	0.12	0.02	—	81	79	118	117	113	107	115	114	118	119	118	119	117	107	112	122	134	125	134	133	146
4 *Siboglinum fiordicum*	0.16	0.17	0.21	—	90	129	125	120	116	121	116	117	128	130	129	125	117	122	127	128	129	142	121	142
5 *Siboglinum ekmani*	0.15	0.16	0.19	0.23	—	121	117	120	116	119	115	117	123	125	123	119	115	120	120	126	129	140	116	133
6 *Escarpia*	0.30	0.30	0.31	0.35	0.33	—	12	29	32	35	34	36	32	36	31	31	35	37	58	126	119	130	117	144
7 Escarpiid n. sp.	0.29	0.29	0.30	0.34	0.31	0.03	—	30	29	32	27	34	30	31	29	27	35	39	52	124	114	126	112	139
8 *Tevnia*	0.30	0.28	0.29	0.33	0.32	0.07	0.07	—	16	17	17	26	43	43	40	39	35	37	70	122	109	133	119	139
9 *Ridgeia* 1	0.30	0.29	0.28	0.33	0.33	0.08	0.07	0.04	—	2	2	17	35	33	32	33	38	37	70	123	106	127	115	138
10 *Ridgeia* 2	0.30	0.29	0.30	0.33	0.32	0.08	0.08	0.04	0.00	—	5	21	37	40	34	36	40	38	73	127	109	134	120	142
11 *Ridgeia* 3	0.28	0.28	0.30	0.31	0.31	0.08	0.06	0.04	0.00	0.01	—	16	35	36	34	32	40	43	68	124	107	131	116	138
12 *Oasisia*	0.30	0.30	0.31	0.32	0.32	0.09	0.08	0.06	0.04	0.05	0.04	—	38	39	41	41	43	47	69	123	108	128	114	139
13 *Lamellibrachia* 1	0.31	0.30	0.32	0.35	0.34	0.08	0.08	0.10	0.09	0.09	0.08	0.09	—	7	11	11	38	42	51	127	115	128	116	146
14 *Lamellibrachia* 2	0.32	0.29	0.31	0.36	0.34	0.09	0.08	0.10	0.08	0.10	0.08	0.09	0.02	—	14	12	36	43	53	127	116	125	118	148
15 *Lamellibrachia* 3	0.32	0.30	0.31	0.35	0.33	0.08	0.07	0.09	0.08	0.08	0.08	0.10	0.03	0.03	—	4	34	35	52	130	118	131	118	150
16 *Lamellibrachia* 4	0.30	0.28	0.31	0.34	0.32	0.08	0.07	0.09	0.08	0.09	0.07	0.10	0.03	0.03	0.01	—	31	35	50	128	116	129	116	148
17 *Riftia* 1	0.32	0.28	0.27	0.32	0.32	0.08	0.08	0.08	0.09	0.10	0.10	0.10	0.09	0.09	0.08	0.07	—	6	58	122	112	125	116	149
18 *Riftia* 2	0.32	0.28	0.29	0.33	0.32	0.09	0.09	0.08	0.09	0.09	0.10	0.11	0.10	0.10	0.08	0.08	0.02	—	62	129	117	133	121	154
19 *Sclerolinum*	0.31	0.31	0.32	0.34	0.32	0.15	0.13	0.18	0.19	0.19	0.17	0.18	0.13	0.13	0.13	0.12	0.15	0.16	—	120	120	129	119	143
20 *Branchipolynoe*	0.38	0.39	0.41	0.38	0.38	0.39	0.38	0.37	0.40	0.39	0.38	0.38	0.39	0.38	0.40	0.39	0.38	0.39	0.36	—	52	126	114	139
21 *Lepidonotopodium*	0.37	0.34	0.38	0.39	0.39	0.35	0.33	0.31	0.32	0.32	0.30	0.32	0.34	0.34	0.35	0.34	0.34	0.35	0.36	0.13	—	126	118	136
22 *Urechis*	0.42	0.38	0.40	0.42	0.43	0.41	0.39	0.42	0.43	0.43	0.41	0.40	0.40	0.38	0.41	0.40	0.40	0.42	0.40	0.38	0.38	—	119	150
23 *Tubifex*	0.35	0.35	0.40	0.34	0.34	0.35	0.34	0.36	0.37	0.37	0.35	0.34	0.35	0.36	0.36	0.35	0.36	0.36	0.36	0.34	0.35	0.36	—	122
24 *Hirudo*	0.43	0.42	0.46	0.44	0.40	0.44	0.42	0.43	0.45	0.44	0.42	0.43	0.45	0.45	0.46	0.45	0.47	0.48	0.43	0.43	0.42	0.46	0.36	—

TABLE 4

Percent of significant tests when comparing relative substitution rates between the two major siboglinid clades

Test type*	Number of tests	Significant results	Percent significant
Between frenulates and vestimentiferan-*Sclerolinum* clade			
18S rDNA	480	331	69.0
16S rDNA	350	47	13.4
Within frenulates			
18S rDNA	150	53	35.3
16S rDNA	50	1	2.0
Within vestimentiferan-*Sclerolinum* clade			
18S rDNA	280	80	28.6
16S rDNA	455	13	2.9

Results of relative rates tests based on an HKY plus gamma model in the HyPhy program. The program is distributed by S. Muse, Department of Statistics, North Carolina State University.

* The 18S comparisons employed all Lophotrochozoan outgroups.

seep tubeworms to be basal to vent tubeworms (but see Williams *et al.*, 1993). This pattern in the evolution of habitat preference roughly proceeds from less reducing to more reducing (greater sulfide and methane availability) environments. A similar evolutionary trend was observed in bathymodiolid mussels (Craddock *et al.*, 1995; Distel *et al.*, 2000). Examination of additional taxa is needed to verify whether this is a general trend in the evolution of vent and seep taxa.

All molecular studies to date (Williams *et al.*, 1993; Black *et al.*, 1997; and Tables 2 and 3) reveal that vestimentiferans exhibit very limited molecular diversity for a group suggested to be several hundred million years old. This lack of diversity may be due to a slowdown in the rate of molecular change (*i.e.*, nucleotide substitution) in vestimentiferans, a recent common origin for extant vestimentiferans, or possibly both. For the present 18S rDNA sequences, vestimentiferans appear to have experienced a significant molecular slowdown relative to the frenulates or other protostome taxa (Table 4; as judged using an HKY plus gamma correction model in the HyPhy software package distributed by S. Muse, Department of Statistics, North Carolina State University). With 16S data, only 13.4% of tests between frenulates and members of the vestimentiferan-*Sclerolinum* clade were significant. Although this value is not statistically significant, it is a greater percentage than is found within either group (~3%), suggesting that a limited rate discrepancy may exist. Similar rate disparities were not observed for COI data (Black *et al.*, 1997), but only one frenulate was used in the comparison. Nonetheless, we concluded that present-day vestimentiferans constitute a young evolutionary group.

In contrast, previous interpretation of Silurian tubeworm fossils (Little *et al.*, 1997) as vestimentiferans suggested that these worms constitute an ancient animal lineage. It is possible that the Silurian tubeworm fossils represent an earlier offshoot from an ancient siboglinid lineage, but this will be impossible to test as the fossils lack the necessary soft-tissue preservation. Additionally, we note that many wormlike invertebrates make tubes. For example, some alvinellid polychaetes observed during our recent expedition to vents along the Southern Eastern Pacific Rise (32°S, 100°W) occupied tubes with diameters comparable to the tubes of mature *Riftia pachyptila*. Many of the alvinellid tubes were partially overgrown by sulfide chimneys, and thus were effectively "fossilized." Although we are not convinced of the interpretation of Silurian fossils as representative of an extant lineage of vestimentiferans, we should point out that specimens from the Cretaceous are convincing (Little *et al.*, 1999). In contrast, all the hydrothermal vent-endemic taxa that have been examined with appropriate molecular tools appear to be from relatively recent radiations (*i.e.*, <100 MY; Black *et al.*, 1997; Peek *et al.*, 1997; Shank *et al.*, 1999; McArthur and Koop, 1999; but see McArthur and Tunnicliffe, 1998, for possible exceptions).

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